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Abstract

After 14 years under conventional plough tillage (CT) or conservation minimum tillage (MT), the soil available Al, Fe, Mn, Cu and Zn (0-5, 5-15 and 15-30 cm layers) and their plant uptake were evaluated during two years in a ryegrass-maize forage rotation in NW Spain (temperate-humid region). The three-way ANOVA showed that trace element concentrations in soil were mainly influenced by sampling date, followed by soil depth and tillage system (35-73 %, 7-58 % and 3-11 % of variance explained, respectively). Excepting for Fe (CT) and Al (CT and MT), the elemental concentrations decreased with depth, the stratification being stronger under MT. For soil available Al, Fe, Mn and Cu, the concentrations were higher in CT than in MT (5-15 and 15-30 cm layers) or were not affected by tillage system (0-5 cm). In contrast, the available Zn contents were higher in MT than CT at the soil surface and did not differ in deeper layers. The concentration of Al, Fe and Cu in crops were not influenced by tillage system, which explain 22 % of Mn variance in maize (CT > MT in the more humid year) and 18 % of Zn variance in ryegrass (MT > CT in both years). However, in the summer crop (maize) the concentrations of Fe, Mn and Zn tended to be higher in MT than in CT under drought conditions, while the opposite was true in the year without water limitation. Therefore, under the studied conditions of climate, soil, tillage and crop rotation, little influence of tillage system on crop nutritive value would be expected. To minimize the potential deficiency of Zn (maize) and Cu (maize and ryegrass) on crop yields the inclusion of these micro-nutrients in fertilization schedule is recommended, as well as liming to alleviate Al toxicity on maize crops.

Keywords: bent-leg subsoiler; fodder maize; *Lolium multiflorum* L.; soil organic carbon; micronutrients;

1. Introduction

In Europe, research on conservation tillage has been mainly focussed in semi-arid areas, where these management practices reduce soil erosion and improve water supply to plants and crop yield (De Vita et al., 2007; Lampurlanés et al., 2002; Martín-Rueda et al., 2007). Consequently, despite its potential environmental and economic advantages, little information is still available for temperate areas on conservation tillage effects in the soil-plant system (Gruber et al., 2012; Soane et al., 2012).

In Spain, around 89,000 ha are cultivated with forage maize (*Zea mays* L.), two-thirds of this surface being located in the northwestern temperate humid zone (MARM, 2009) where it is the most common crop under conservation tillage, mainly in maize-italian ryegrass rotations. For this crop rotation, conservation tillage has economic and timeliness advantages without detrimental effect on forage yields (Bueno et al., 2007). Moreover, conservation practices improved the physical, chemical and biological properties in the topsoil layer under this forage rotation (Bueno et al., 2006; Díaz-Raviña et al., 2005; Gómez-Rey et al., 2012).

Compared with conventional ploughed fields, soil disturbance under conservation tillage management (without soil inversion) was reduced. As a consequence, the interaction of soil with crop residues and fertilizers decreased, leading to changes in the distribution of nutrients along the soil profile, with higher levels in the topsoil (Edwards et al., 1992; Franzluebbers and Hons, 1996; López-Fando and Pardo, 2009; Martín-Rueda et al., 2007; Wright et al., 2007). Moreover, timing of nutrient release was also affected (Houx et al., 2011) with possible effects on nutrient availability to plants and, therefore, on nutrient disequilibrium and fertilizer requirements

(Holanda et al., 1998; Yin and Vyn, 2004). However, until present time, published studies on the effect of tillage on trace elements in soils and crops are scarce (Lavado et al., 2001; López-Fando and Pardo, 2009; Stanislawska-Glubiak and Korzeniowska, 2011; Stanislawska-Glubiak et al., 2009), and the few available reports showed contradictory results probably due to the interaction with soil type, crop species and fertiliser practices (Watson et al., 2012). While Westermann and Sojka (1996) did not find differences in soil trace concentration among tillage systems, other studies reported higher levels of Mn and Zn under conservation tillage than under ploughing tillage (Edwards et al., 1992; Franzluebbers and Hons, 1996; Loke et al., 2013; López-Fando and Pardo, 2009; Martín-Rueda et al., 2007; Rhoton, 2000) and the opposite tendency for Fe (López-Fando and Pardo, 2009; Rhoton, 2000) and Cu (Loke et al., 2013; Rhoton, 2000). Although tillage practice can modify trace element concentrations in crops (Stanislawska-Glubiak and Korzeniowska, 2009), no differences on Mn, Cu and Zn concentration have also been reported for barley straw (Martín-Rueda et al., 2007), sorghum (de Santiago et al., 2008) and flax (Grant et al., 2010). These contrasting results could be related with soil and crop characteristics, but also with meteorological conditions during the growing season because higher crop levels of Cu, Zn, Mn and B have been reported for zero-tillage under drought conditions and for ploughing tillage when water supply was good (Stanislawska-Glubiak et al., 2009).

Accordingly, the working hypothesis is that, compared with ploughing tillage, conservation tillage changed the soil trace elements distribution with depth and affected yields and Al, Fe, Mn, Cu and Zn contents of crops. Thus, present study aimed to evaluate the long-term effect of two tillage practices (conventional and minimum tillage during 14 years) on soil trace elements levels, crop yields and plant nutrient contents in a ryegrass-maize forage rotation.

2. Material and methods

2.1. Site description

The experimental field was located in the Gayoso-Castro farm (43º 06′N, 7º 27′ W, 420 m a.s.l.) at Castro de Ribeiras de Lea (Galicia, NW Spain). The area has a temperate and rainy climate. During the study period (October 2006-October 2008), at the meteorological stations of As Rozas, Rubiás and Lugo, located within a radius of 17 km from the farm and at similar altitude, rainfall mainly occurred in the October to June period (Fig. 1). The rainiest month was October 2006 (Meteogalicia, 2013). The soil is a Gleyic Phaeozem (IUSS Working Group, 2006) developed over sandy-clayey deposits, with sandy loam topsoil (around 70 % of sand), acidic pH_{H2O} (about 5.5) and an organic C content of 3.1-7.9 g kg^{-1}.

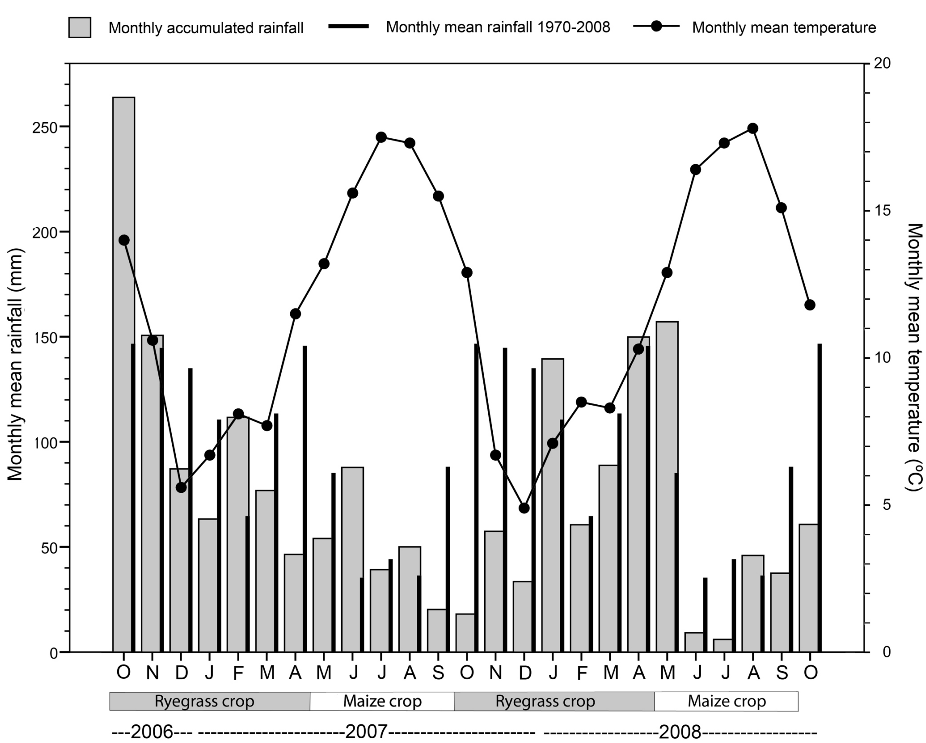

Fig. 1. Monthly mean temperature (ºC, points connected by a line) and rainfall (mm, wide grey bars) during the growing season of ryegrass and maize. For comparison, the vertical dark lines show the monthly mean precipitation during the 1970–2008 period.

Since 1994, a rotation of silage maize (*Zea mays* L.) and Italian rye-grass (*Lolium multiflorum* L.) has been annually cultivated in two adjacent areas with different tillage system: conventional plough tillage (CT) and conservation minimum tillage (MT). Each area was divided in nine replicate plots (4 m x 3 m; 1 m separation). Maize was sown in rows 0.75 m apart (appr. 95,000 plants ha^{-1}, 4 rows per plot) in late May and harvested in late September, while rye-grass was sown in rows 0.17 m apart (40 kg ha^{-1}, 17.5 rows per plot) in late October and harvested in early May. In the MT treatment, before maize sowing, the adventitious vegetation was destroyed with glyphosate (36 %, at a dose of 5 L ha^{-1}). In the MT system, after 8-yr of no-tillage the management was changed to minimum tillage to revert the problem of increasing soil compaction and decreasing maize emergence and the soil was loosened with a bent-leg subsoiler to a depth of 30 cm. In the CT treatment, the soil was ploughed at 25-30 cm with a reversible plough twice a year (May and October), to incorporate crop residues and to prepare seed bed. Further agrochemical treatments were similar for both tillage systems. During the maize cultivation, the plots were treated with herbicides (33 % acetachlor and 16.5 % atrazine, 4 L ha^{-1}), insecticide (48 % clorpiriphos, 0.33 L ha^{-1}) and NPK 9-18-27 fertilizer (N: 63 kg ha^{-1}; P_2O_5: 126 kg ha^{-1}; K_2O: 189 kg ha^{-1}). During the rye-grass cultivation, the plots received NPK fertilizer in early October (N: 27 kg ha^{-1}; P_2O_5: 54 kg ha^{-1}; K_2O: 81 kg ha^{-1}) and NH_4NO_3 fertilizer in early March (N: 81 kg ha^{-1}).

2.2. Soil and plant sampling

Soil samples were collected just after rye-grass (May 2007 and 2008) and maize (October 2007 and 2008) harvesting. In each plot, soil (0-5, 5-15 and 15-30 cm depth) was taken with a stainless steel probe (4 cm internal diameter) from 8 points uniformly distributed between the rows; afterwards it was thoroughly mixed to obtain a composite sample per plot, sieved (< 2 mm) and air dried. Soil water-holding capacity was determined in a Richards membrane-plate extractor at a pressure of 10 kPa. Soil texture was determined (on the < 2 mm soil fraction) by the international mechanical analysis method.

Plant sampling to determine biomass and respective nutrient contents was performed in May (rye-grass) and in October (maize) of 2007 and 2008. For calculating the aboveground biomass, all plants of the plot were cut at the base and weighted. Ryegrass yielded 3158 (CT) and 3114 kg ha^{-1} (MT) in 2007 and 4425 (CT) and 4903 kg ha^{-1} (MT) in 2008. For maize, in 2007 the productions were 6613 (CT) and 6461 kg ha^{-1} (MT), while in 2008 they were 4755 (CT) and 7313 kg ha^{-1} (MT).

For chemical analysis, only plants from the plot centre (75 cm inward from the edge) were considered, which were homogenized and crushed in situ, and a subsample was taken for chemical analysis. The subsample was dried at 60 ºC during 10 h and newly crushed to a size of less than 4 mm.

2.3. Chemical analysis

The dry matter content of soils and plant material was assessed by oven-drying subsamples at 110 ºC to constant weight. Soil total C was measured on finely ground samples (< 100 μm) with an elemental analyser (Carlo Erba CNS 1508). For available trace elements analyses, soils (10 g) were shaken for 2 h with an extracting solution of 1 M NH_4Ac and 0.005 M DTPA (1:5 soil to solution ratio); the extracts were filtered through cellulose filter paper and then analysed for trace elements (Al, Fe, Mn, Cu and Zn) by simultaneous ICP-OES (Varian Vista Pro, Mulgrave, Australia).

The plant material was finely ground (< 100 μm) for chemical analysis. For determining the total nutrient content of plant material, a subsample (500 mg) was digested for 55 min with 8 mL of 65 % HNO_3 and 25 mL of 30 % H_2O_2 in Teflon containers in a high performance microwave digestion unit (Milestone 1200 Mega, Sorisole, Italy). Once cooled, the solutions were filtered through quantitative cellulose filter paper, transferred to 25 mL volumetric flasks, and made to volume with water. The total trace elements content (Al, Fe, Mn, Cu and Zn) was measured by simultaneous ICP-OES.

Analytical-grade chemicals were obtained from Merck Chemical Co., quantitative cellulose filter paper from Filter-laboratory (1242, 90-mm diameter) and all aqueous solutions were prepared with type I water (ASTM 2008). All analyses were carried out in duplicate and the mean of both analyses was used in the statistical procedure.

2.4. Calculation and statistical analysis

Data of soil and plant variables were statistically analysed by three-way and two-way ANOVA, respectively, with tillage system, soil depth and sampling date as factors for extractable soil nutrients and with tillage system and date for concentration and content in plants. After checking the equality of variances among groups with Levene's test, significant differences among their means were established at $P< 0.05$ using the Bonferroni's test for multiple comparisons. With unequal variances, the original data were subjected to the Tukey's ladder of power, or to Cox-Box transformations, to obtain equality of variances and then significant differences among the mean groups were established at $P< 0.05$ using the Bonferroni's test. The proportion of the variation accounted for each factor or interaction in the ANOVA was determined by the partial eta-squared ($\eta 2$) statistic. Statistical procedures were performed using SPSS 15.0 for Windows.

3. RESULTS

No differences among sampling dates were found for soil texture, water holding capacity (WHC) and organic C. Neither tillage nor depth have significant effects on soil texture (Table 1), while both factors have intense effects on WHC (73-75% of variance explained) but their strong interaction (63% of variance explained) showed that WHC decreased significantly with soil depth only in MT. The tillage system explained 7.2% of the variance of soil C concentration, average values being significantly higher under MT than CT, but only in the top soil layer (significant tillage x depth interaction). Depth explained 34% of the variance of soil C, which was more stratified under MT than under CT (Table 1).

Table 1. Particle size, water holding capacity (WHC) and organic C content of the soil (< 2 mm) collected under conventional (CT) or minimum (MT) tillage; values are mean ± standard deviation for the four sampling dates.

	CT			MT		
	0-5 cm	5-15 cm	15-30 cm	0-5 cm	5-15 cm	15-30 cm
Sand (g kg^{-1} dw)	69 ± 3 ^{aA}	66 ± 4 ^{aA}	72 ± 6 ^{aA}	71 ± 1 ^{aA}	72 ± 5 ^{aA}	71 ± 2 ^{aA}
Silt (g kg^{-1} dw)	13 ± 1 ^{aA}	16 ± 2 ^{aA}	12 ± 6 ^{aA}	13 ± 1 ^{aA}	10 ± 8 ^{aA}	13 ± 1 ^{aA}
Clay (g kg^{-1} dw)	17 ± 2 ^{aA}	19 ± 3 ^{aA}	16 ± 1 ^{aA}	16 ± 1 ^{aA}	19 ± 3 ^{aA}	16 ± 3 ^{aA}
WHC (g H_2O kg^{-1} dw)	315 ± 16 ^{bA}	318 ± 22 ^{bA}	306 ± 3 ^{aA}	392 ± 17 ^{aA}	358 ± 2 ^{aB}	310 ± 12 ^{aC}
WHC (Mg H_2O ha^{-1})	83 ± 4 ^{b}	169 ± 12 ^{b}	243 ± 2 ^{a}	104 ± 5 ^{a}	190 ± 1 ^{a}	246 ± 10 ^{a}
Organic C (g kg^{-1} dw)	52.8 ± 6.1 ^{bA}	51.0 ± 4.4 ^{aA}	47.5 ± 5.0 ^{aB}	62.0 ± 7.3 ^{aA}	50.8 ± 5.0 ^{aB}	48.2 ± 5.7 ^{aB}

Different lowercase letters indicate significant differences ($p<0.05$) between tillage systems for the same soil depth. Different uppercase letters indicate significant differences ($p<0.05$) among depths for the same tillage system; due to the different thickness of soil layers, this comparison was not done for WHC expressed as Mg H_2O ha^{-1}.

3.1. Soil available trace elements

The three-way ANOVA showed that the levels of available Al were significantly ($P< 0.001$) affected by tillage, soil depth and sampling date, that explained 11 %, 7 % and 66 % of variance, respectively, all two order interactions being also significant (Table 2). The tillage x depth interaction showed that soils under CT have significantly more available Al than those under MT at all depths except in the uppermost layer (for which no a clear trend was found, see Table 2) and the last sampling date (Fig. 2). While no significant changes with depth were found under CT, under MT the available Al concentrations decreased in the order 0-5 cm > 5-15 cm ≈ 15-30 cm (Table 2).

While the tillage system explained 12 % of the variation of available Fe, soil depth and sampling date accounted for 25 % and 73 %, respectively. All two order interactions have significant but weak effects on available Fe concentration (Table 2) which did not differ between tillage systems (at 0-5 cm depth) or it was higher under CT than under MT (at 5-15 and 15-30 cm depth). Under CT the concentration of available Fe was homogeneous in the 0-30 cm soil layer, while under MT a stratification was observed, decreasing in the order 0-5 cm > 5-15 cm ≈15-30 cm (Fig. 3).

Table 2. Mean values ± standard deviation (n=9) of the soil extractable Al, Fe, Mn, Cu and Zn (mg kg^{-1}) and results of the three-way ANOVA with tillage system (CT: conventional tillage, MT: minimum tillage), soil depth and sampling date as factors.

	Al	Fe	Mn	Cu	Zn
Tillage					
CT	409.8 ± 101.3 ^{a}	179.4 ± 49.9 ^{a}	5.33 ± 2.48 ^{a}	0.16 ± 0.06 ^{a}	0.47 ± 0.23 ^{b}
MT	371.8 ± 68.75 ^{b}	162.9 ± 41.3 ^{b}	4.74 ± 2.45 ^{b}	0.13 ± 0.04 ^{b}	0.52 ± 0.24 ^{a}
Depth					
0-5 cm	406.2 ± 82.5 ^{a}	186.5 ± 48.0 ^{a}	7.06 ± 2.71 ^{a}	0.16 ± 0.06 ^{a}	0.67 ± 0.29 ^{a}
5-15 cm	382.3 ± 90.0 ^{b}	166.7 ± 42.5 ^{b}	4.33 ± 1.50 ^{b}	0.14 ± 0.05 ^{b}	0.43 ± 0.14 ^{b}
15-30 cm	381.2 ± 90.7 ^{b}	160.1 ± 45.0 ^{b}	3.66 ± 1.55 ^{c}	0.13 ± 0.04 ^{b}	0.38 ± 0.14 ^{c}
Date					
May-2007	490.6 ± 65.2 ^{a}	224.5 ± 35.7 ^{a}	6.05 ± 2.18 ^{a}	0.18 ± 0.04 ^{a}	0.63 ± 0.31 ^{a}
October-2007	372.1 ± 82.9 ^{b}	147.8 ± 31.4 ^{c}	5.12 ± 2.62 ^{b}	0.15 ± 0.05 ^{b}	0.46 ± 0.23 ^{b}
May-2008	388.7 ± 45.3 ^{b}	178.7 ± 32.8 ^{b}	6.11 ± 2.28 ^{a}	0.16 ± 0.05 ^{ab}	0.52 ± 0.14 ^{a}
October-2008	311.6 ± 39.8 ^{c}	133.4 ± 21.6 ^{d}	2.86 ± 1.07 ^{c}	0.09 ± 0.03 ^{c}	0.36 ± 0.15 ^{c}
Tillage x Depth					
CTx0-5 cm	413.8 ± 99.3 ^{aA}	184.1 ± 54.3 ^{aA}	6.93 ± 2.96 ^{aA}	0.18 ± 0.07 ^{aA}	0.59 ± 0.34 ^{bA}
CTx5-15 cm	402.7 ± 104.9 ^{aA}	177.0 ± 45.3 ^{aA}	4.82 ± 1.70 ^{aB}	0.16 ± 0.07 ^{aAB}	0.44 ± 0.14 ^{aB}
CTx15-30 cm	409.0 ± 102.3 ^{aA}	177.0 ± 50.8 ^{aA}	4.21 ± 1.74 ^{aC}	0.14 ± 0.05 ^{aB}	0.38 ± 0.10 ^{aB}
MTx0-5 cm	398.5 ± 61.9 ^{aA}	188.9 ± 41.3 ^{aA}	7.20 ± 2.47 ^{aA}	0.15 ± 0.04 ^{aA}	0.75 ± 0.22 ^{aA}
MTx5-15 cm	361.9 ± 67.7 ^{bB}	155.9 ± 36.9 ^{bB}	3.83 ± 1.07 ^{bB}	0.13 ± 0.03 ^{bB}	0.42 ± 0.14 ^{aB}
MTx15-30 cm	354.9 ± 70.1 ^{bB}	143.6 ± 31.4 ^{bB}	3.14 ± 1.14 ^{bC}	0.12 ± 0.03 ^{aB}	0.38 ± 0.17 ^{aB}
η_p^2 Tillage	0.111 ***	0.121 ***	0.072 ***	0.044 **	0.035 **
η_p^2 Depth	0.073 ***	0.251 ***	0.584 ***	0.143 ***	0.442 ***
η_p^2 Date	0.664 ***	0.731 ***	0.636 ***	0.523 ***	0.353 ***
η_p^2 Tillage x Depth	0.038 *	0.127 ***	0.102 ***	0.005 n.s.	0.133 ***
η_p^2 Tillage x Date	0.297 ***	0.163 ***	0.117 ***	0.061 **	0.045 *
η_p^2 Depth x Date	0.151 ***	0.082 *	0.140 ***	0.038 n.s.	0.069 *
η_p^2 Tillage x Depth x Date	0.038 n.s.	0.041 n.s.	0.064 *	0.044 n.s.	0.200 ***

For the Tillage, Depth and Date factors, different lowercase letters in the same column indicate significant differences ($p < 0.05$). For the tillage x depth interaction, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depths for the same tillage system. $p<0.05$; ** $p< 0.01$; *** $p< 0.001$; n.s. not significant .

As for Fe, the three-way ANOVA (Table 2) showed that most part of the variance of available Mn was explained by soil depth (58 %) and sampling date (64 %), with only a reduced effect of tillage system (7.2 %). All two and three order interactions were significant, but they explained only 6-14 % of the variance (Table 2). The available Mn levels did not differ between tillage systems at 0-5 cm depth except after the second maize crop and were higher in CT than in MT at 5-15 and 15-30 cm depth (Fig. 4). Manganese concentrations in the 0-30 cm layer were not homogenized by ploughing in CT and in both tillage systems the available Mn was stratified and decreased progressively along the depth profile (0-5 cm > 5-15 cm > 15-30 cm) (Fig. 4 and Table 2).

The main factor influencing the available Cu content was the sampling date, followed by soil depth and tillage system (52 %, 14 % and 4.4 % of variance explained, respectively) and the only significant interaction was that of tillage x date (6.1 % of variance explained) (Table 2). As for the other trace elements, the available Cu levels were higher under CT than MT, but only at the 5-15 cm soil layer from May samplings (Fig. 5). Irrespectively of the tillage system, the available Cu concentrations where higher in the 0-5 cm than in the 15-30 cm soil layer. Values in the 5-15 cm layer were intermediate (in CT system) or lower (in MT system) than in the uppermost layer.

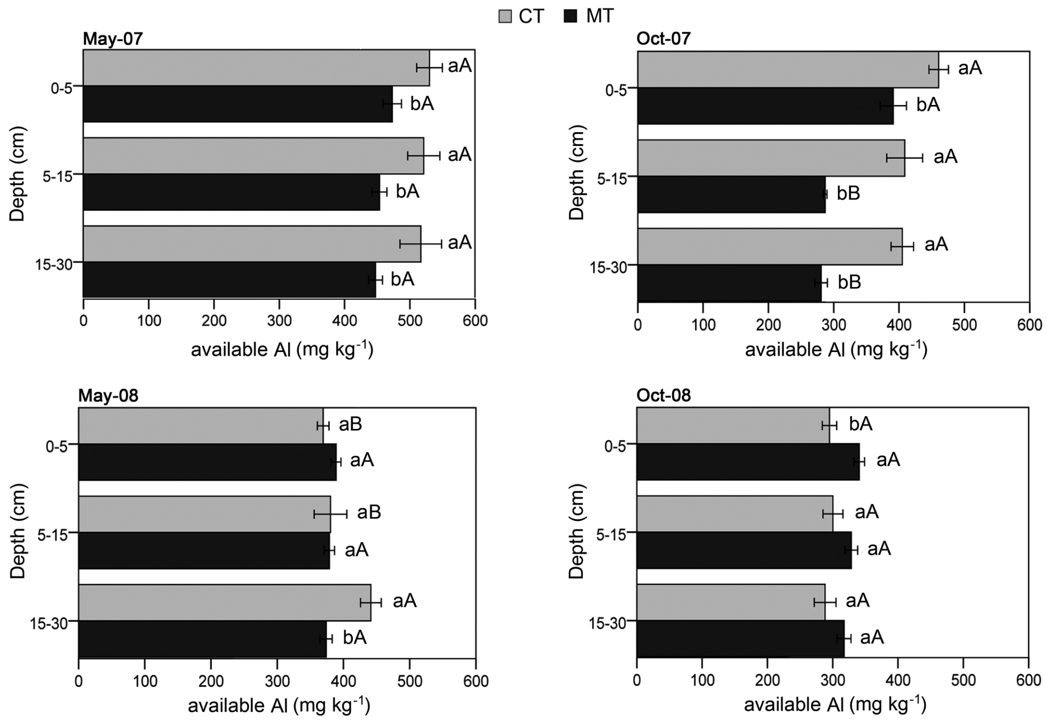

Fig. 2. Available Al concentration (g kg^{-1}) in the soil collected under conventional (CT) or minimum (MT) tillage at different sampling dates. For each sampling date, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n = 9, P < 0.05). Bars represent ± standard deviation.

For the available Zn, the variance explained decreased in the order soil depth (44 %), sampling date (35 %) and tillage system (3.5 %). All two- and three-order interactions have a significant effect on available Zn levels, but explained only 4-20 % of variance (Table 2). Contrarily to the other trace elements, the concentration of available Zn were higher in MT than in CT (at 0-5 cm depth, except in the first sampling date; Fig. 6), or were not affected by tillage system (at 5-15 and 15-30 cm soil layers). Under CT and MT the concentration of available Zn was higher in the soil surface (0-5 cm) than in the 5-10 and 10-15 cm layers (Table 2 and Fig. 6).

3.2. Crop uptake of trace elements

According with the two-way ANOVA, neither the Al concentration nor the Al content exported with the maize crops was affected by any of the studied factors (Table 3). For ryegrass, the sampling date explained 60 % and 42 % of the variance ($P< 0.001$) in Al concentration and uptake, respectively, with higher values in the crop of 2007 than in that of 2008 (Fig. 7).

At harvesting, Fe concentration and content in ryegrass was unaffected by sampling date and tillage system, but in maize significant effects of date (Fe concentration) and the date x tillage system interaction (Fe concentration and content) were found (16 % to 24 % of variance explained; $P< 0.05$ to $P< 0.01$; Table 3), with higher values under CT in 2007 and MT in 2008 (Fig. 7).

While date was the only factor with influence on the Mn concentration and Mn content exported with the ryegrass crops (27 % and 72 % of variance explained, respectively, $P< 0.01$ to $P< 0.001$; Table 3), for the maize crops a slight effect of tillage system and the date x tillage system was observed (22-23 % of variance explained, $P< 0.001$; Table 3). For ryegrass the highest values were recorded in 2008 and for maize higher values were found under CT in 2007 and MT in 2008 (Fig. 7).

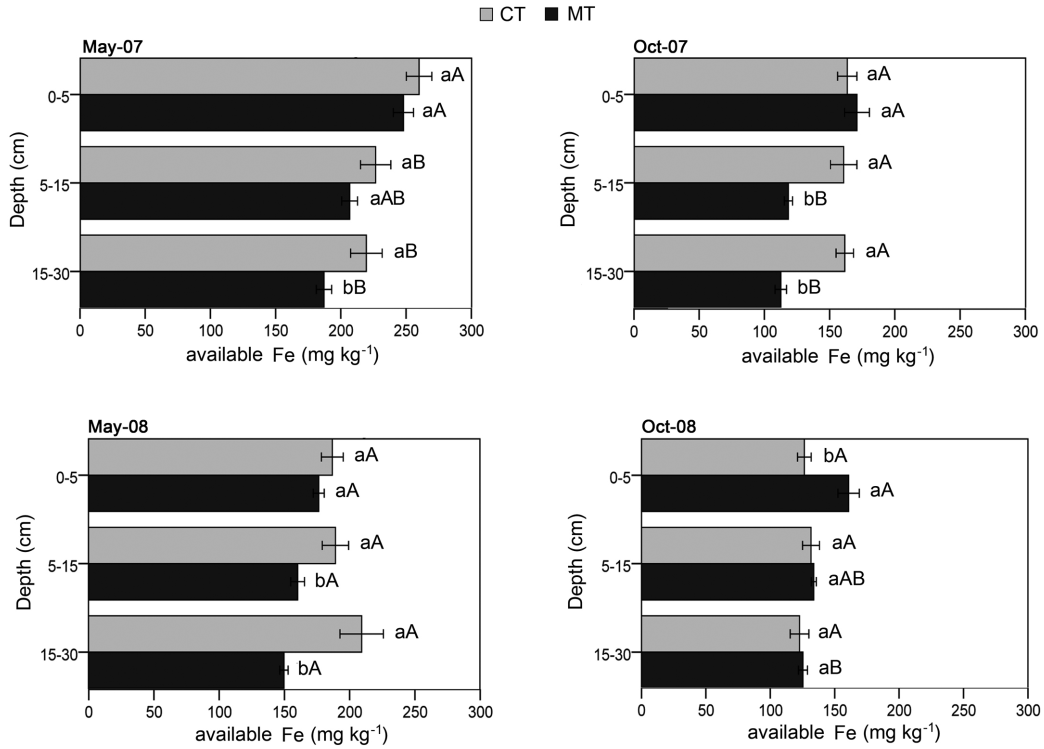

Fig. 3. Available Fe concentration (mg kg^{-1}) in the soil collected under conventional (CT) or minimum (MT) tillage at different sampling dates. For each sampling date, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n = 9, P < 0.05). Bars represent ± standard deviation.

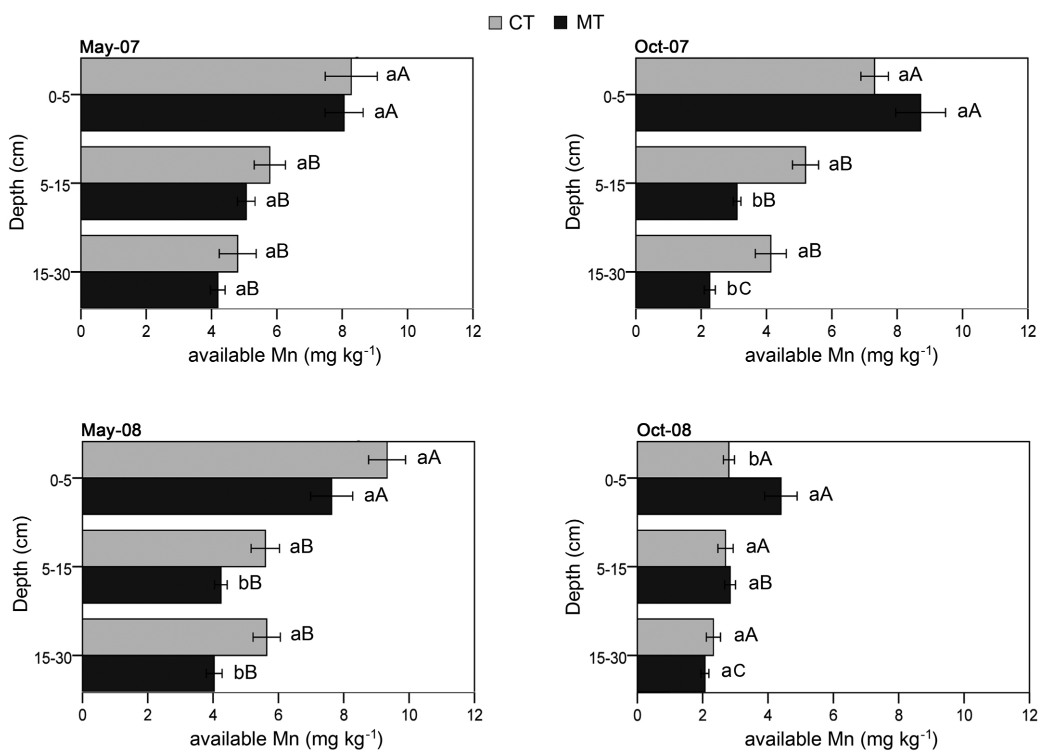

Fig. 4. Available Mn concentration (mg kg^{-1}) in the soil collected under conventional (CT) or minimum (MT) tillage at different sampling dates. For each sampling date, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n = 9, P < 0.05). Bars represent ± standard deviation.

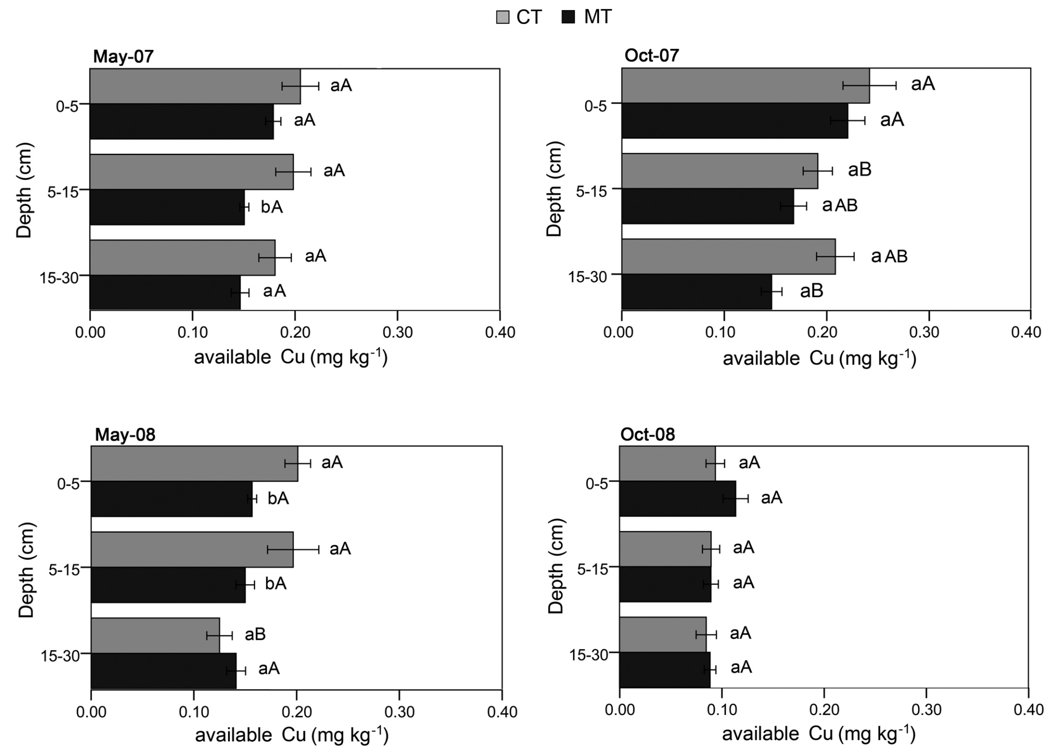

Fig. 5. Available Cu concentration (mg kg^{-1}) in the soil collected under conventional (CT) or minimum (MT) tillage at different sampling dates. For each sampling date, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n = 9, P < 0.05). Bars represent ± standard deviation.

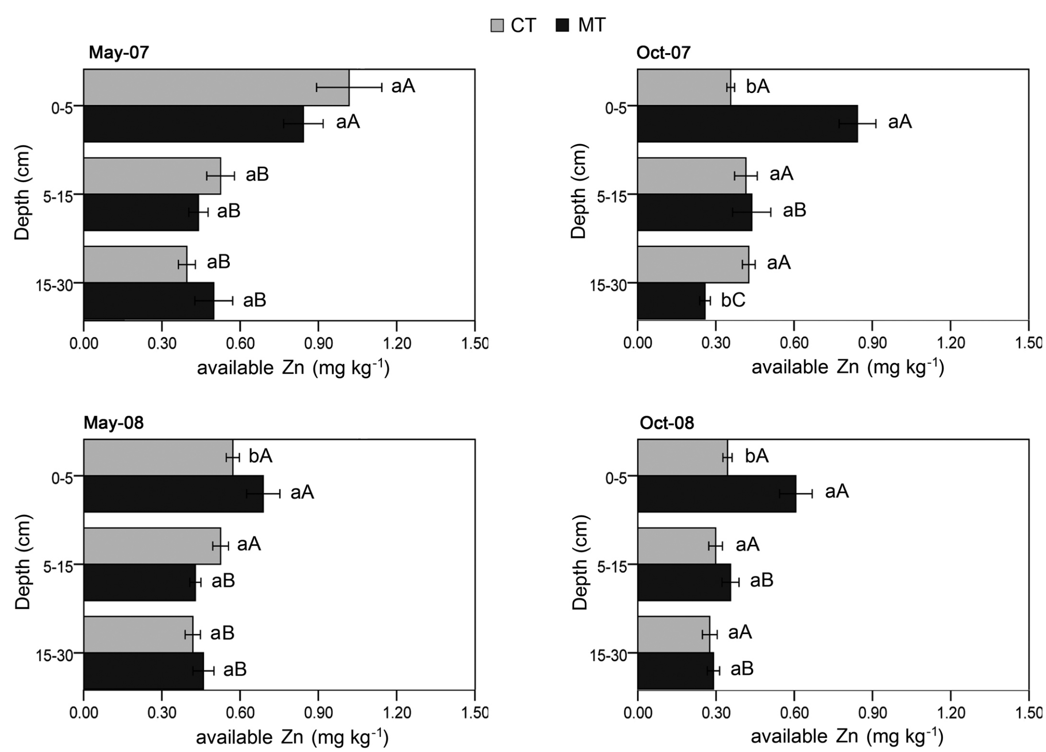

Fig. 6. Available Zn concentration (mg kg^{-1}) in the soil collected under conventional (CT) or minimum (MT) tillage at different sampling dates. For each sampling date, lowercase letters indicate significant differences between tillage systems for the same soil depth and uppercase letters indicate significant differences among depth for the same tillage system (n = 9, P < 0.05). Bars represent ± standard deviation.

Table 3. Results of the two-way ANOVA for the concentration and uptake of Al, Fe, Mn, Zn and Cu in the aboveground biomass of ryegrass and maize with date and tillage system as factor.

			Date		Tillage system		Date x tillage	
			partial η^2	p	partial η^2	p	partial η^2	p
Al	concentration	ryegrass	0.595	***	0.125	n.s.	0.068	n.s.
		maize	0.050	n.s.	0.041	n.s.	0.009	n.s.
	uptake	ryegrass	0.419	***	0.153	n.s.	0.117	n.s.
		maize	0.030	n.s.	0.001	n.s.	0.094	n.s.
Fe	concentration	ryegrass	0.111	n.s.	0.030	n.s.	0.013	n.s.
		maize	0.160	*	0.002	n.s.	0.244	**
	uptake	ryegrass	0.067	n.s.	0.032	n.s.	0.014	n.s.
		maize	0.000	n.s.	0.001	n.s.	0.155	*
Mn	concentration	ryegrass	0.265	**	0.063	n.s.	0.036	n.s.
		maize	0.012	n.s.	0.215	**	0.227	**
	uptake	ryegrass	0.715	***	0.000	n.s	0.003	n.s
		maize	0.009	n.s	0.006	n.s	0.159	*
Zn	concentration	ryegrass	0.046	n.s.	0.181	*	0.012	n.s.
		maize	0.229	**	0.031	n.s.	0.013	n.s.
	uptake	ryegrass	0.181	*	0.196	**	0.07	n.s
		maize	0.000	n.s	0.001	n.s	0.115	*
Cu	concentration	ryegrass	0.000	n.s	0.078	n.s	0.001	n.s
		maize	0.002	n.s	0.032	n.s	0.041	n.s
	uptake	ryegrass	0.016	n.s	0.048	n.s	0.046	n.s
		maize	0.001	n.s	0.022	n.s	0.000	n.s

* p<0.05; ** p< 0.01; *** p< 0.001; n.s. not significant

While date was the only factor with influence on the Mn concentration and Mn content exported with the ryegrass crops (27 % and 72 % of variance explained, respectively, P< 0.01 to P< 0.001; Table 3), for the maize crops a slight effect of tillage system and the date x tillage system was observed (22-23 % of variance explained, P< 0.001; Table 3). For ryegrass the highest values were recorded in 2008 and for maize higher values were found under CT in 2007 and MT in 2008 (Fig. 7).

None of the studied factors had significant effects on Cu concentration and Cu content in the harvested biomass (Table 3).The two-way ANOVA showed slight effects of date and tillage system on the Zn concentration and Zn content only in ryegrass crops (18-20 % of variance explained; P< 0.05 to P< 0.01, Table 3). Higher values were always found under MT, although differences with CT were not significant in 2007. The amount of Zn exported with the crop, jointly considering both tillage systems, was higher in 2008 than in the precedent year (Fig. 7).

4. DISCUSSION

4.1. Soil available trace elements

As Houx et al. (2011) highlighted, tillage and rotation effects on soil available Al have received little attention in published research. Although these authors did not find differences between no-tillage and CT for the available Al, our results showed that soils under CT have more available Al than those under MT except in the uppermost layer. In the studied plots, the Al concentrations were homogenized by ploughing in CT, while in MT the uppermost layer has the highest values. It is well documented that plants can reduce Al availability by

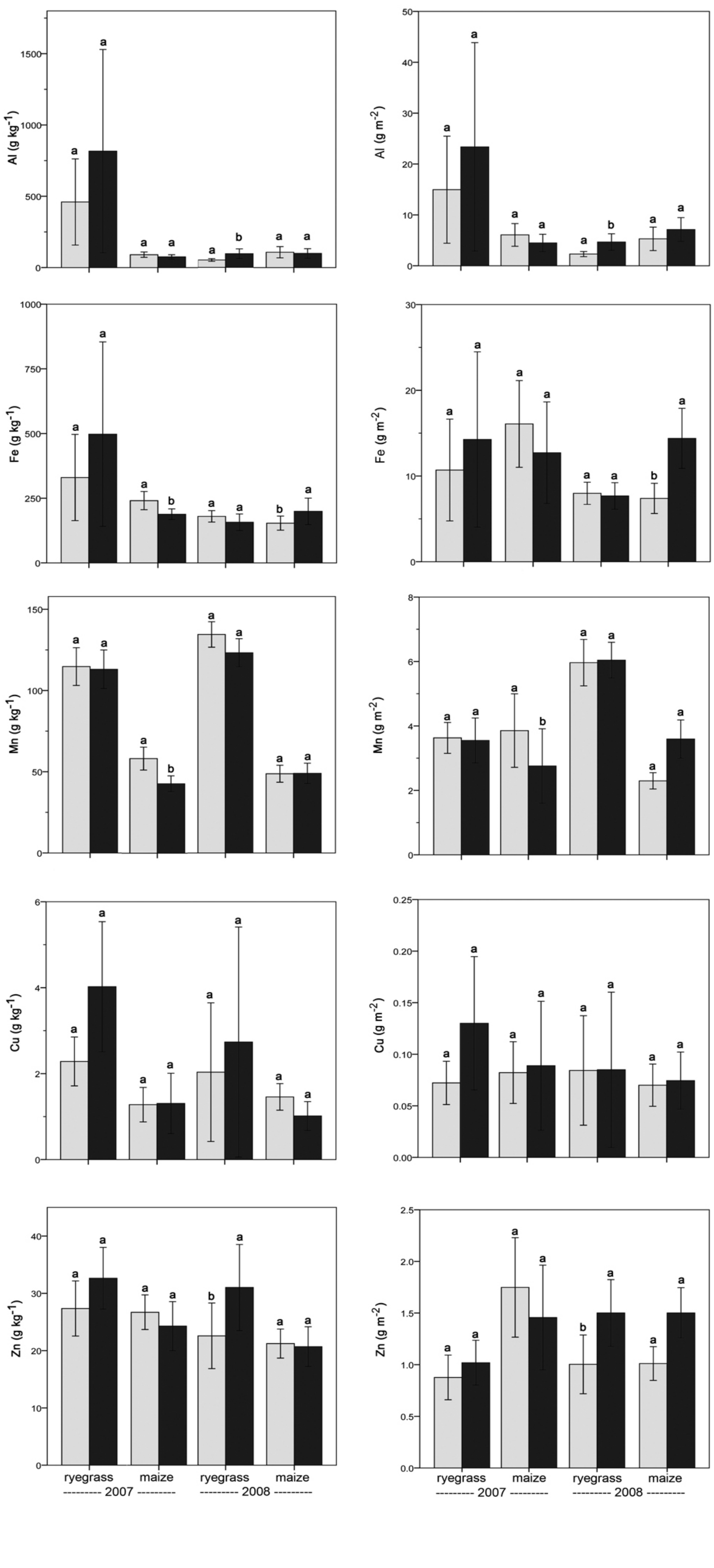

Figure 7. Plant concentration (g kg^{-1}) and uptake (g m^{-2}) of Al, Fe, Mn, Cu and Zn for ryegrass and maize growing under conventional (CT) or minimum (MT) tillage. For each growing season and a given crop, different letters indicate differences between tillage systems (n= 9; p< 0.05). Bars represent standard deviation.

extracellular precipitation or detoxification by complexation with chelating root exudates (organic acid anions) or binding to mucilage (Barceló and Poschenrieder, 2002; Zheng, 2010). Therefore, jointly considering all depths, the reduced Al availability under MT could be related with its greater cover of adventitious vegetation.

Contrasting results have been reported on the relationships between tillage system and soil Fe availability. While some researchers found higher Fe availability under conservation tillage than under conventional tillage (Franzluebbers and Hons, 1996; Houx et al., 2011; Martin-Rueda et al., 2007), other authors observed the opposite tendency (Lavado et al., 1999; Rhoton, 2000) or no differences due to tillage (de Santiago et al., 2008; Loke et al., 2013). Like those of the former authors, our results showed that the available Fe levels were slightly affected by the tillage system, the effect being depth dependent as previously reported Martín-Rueda et al. (2007), with no difference in the 0-5 cm layer and higher values under CT than under MT in the other soil layers. This result was due to the different pattern of available Fe with depth: a progressive decrease under MT, as also found Lavado et al. (1999), and homogeneous levels due to ploughing under CT. In the same way, Wright et al. (2007) indicated that the higher macro- and micronutrient levels in surface relative to subsurface soils were likely a result of greater decomposition of soil organic matter and crop residues, releasing inorganic nutrients and contributing to accumulation at 0–15 cm.

The tillage system has only a reduced effect on soil available Mn, with most part of significant differences being favourable to CT. Our results were partially consistent with those of some authors that reported either no differences due to tillage (de Santiago et al., 2008; Loke et al., 2013; Stanislawska-Glubiak et al., 2009) or higher Mn availability under CT (Houx et al., 2011; Lavado et al., 1999), but contrast with other showing the opposite tendency (Edwards et al., 1992; Franzluebbers and Hons, 1996; López-Fando and Pardo, 2009; Martín-Rueda et al., 2007). Unlike the other studied trace elements, the concentration of available Mn was stratified both under MT and (to a lesser extend) CT as also found Franzluebbers and Hons (1996) and Wright et al. (2007). In our plots, Mn stratification could be due to Mn correlation with SOC levels ($r=0.458$, $P< 0.01$; $n=212$) or to losses from the subsurface soil layers because the water table was near the soil surface several months a year, as indicated by the soil type (Gleyic Phaeozem), and Mn solubility increases under acidic and reducing conditions (Mortvedt, 1983).

A lack of differences between conventional and minimum tillage, as we found for most soil depths, is the more frequently reported result for Cu availability under contrasting tillage systems (de Santiago et al., 2008; Edwards et al., 1992; Houx et al., 2011), although higher available Cu under CT (Lavado et al., 1999) or MT (Franzluebbers and Hons, 1996; Martin-Rueda et al., 2007) have also been reported. The extractable Cu usually increases with depth to 0.2-0.3 m and decreases thereafter (Edwards et al., 1992; Franzluebbers and Hons, 1996; Wright et al., 2007), or shows no trends to stratify (Lavado et al., 1999). Contrastingly, we found a slight accumulation in the uppermost soil layer under both tillage systems; as soil C content was stratified under MT but not under CT, this result cannot be related with the C content but with a greater decomposition of soil organic matter and crop residues at the soil surface as reported by Wright et al. (2007).

Among the trace elements considered in the present study, Zn was with Cu the less affected by the tillage system which explained only 3.5 % of Zn variance. Singularly, Zn concentrations were never higher in CT than in MT, as also found most researchers (de Santiago et al., 2008; Edwards et al., 1992; Franzluebbers and Hons, 1996; Houx et al., 2011; Loke et al., 2013; Martín-Rueda et al., 2007; Stanislawska-Glubiak et al., 2009; Wright et al., 2007). Agreeing with Franzluebbers and Hons (1996) and Wright et al. (2007), extractable Zn was greater near the soil surface and decreased with depth in both MT and CT, leading to a stratification related with (but greater than) that observed for organic C ($r=0.482$, $P< 0.01$; $n=212$).

4.2. Crop uptake of trace elements

The lack of sound effects of tillage systems on the trace elements exported with crops, expressed as plant concentrations or as amount per cultivated surface, suggested that no differences in crop nutritive value due to tillage system would be expected under the studied conditions of climate, soil, tillage and crop rotation. However, partially agreeing with Stanislawska-Glubiak and Korzeniowska (2009), the levels of Fe, Mn and Zn (but not Cu) in maize tended to be higher in MT than CT under drought conditions (2008), while the opposite trend was observed the year without water limitation (2007). It must be highlighted that precipitation was much more evenly distributed during the maize growth period in 2007 (39 to 88 mm month^{-1}, except September with 20 mm) than in 2008 (6 to 157 mm month^{-1}) with a drought period (15 mm of accumulated rainfall) in June-July, during a critical stage for the growth of the young maize plants. In the studied plots, due to the sandy loam texture, soil water holding capacity largely rely on SOM content which is significantly higher under MT than under CT. As a consequence, compared with CT, the plough layer of MT can store 9.2% more water (495 and 540 Mg H_2O ha^{-1}, respectively); the importance of this additional water supply in MT (equivalent to 4.5 mm of rainfall) was evident when compared with the 15 mm of rainfall in June-July 2008. Moreover, under MT crop residues were left on soil surface as mulch, with a well know water conserving effect due to an increase of water retention in the soil profile and a reduction of evaporation losses (Erenstein, 2002). Although the water conserving effect of crop residues mulch may have little tangible effect on yields in "normal" years, it is particularly beneficial in dry years with the important benefit of reducing productive risk and yield oscillations (Erenstein, 2002). Therefore, the contrasting results observed for Fe, Mn and Zn in 2007 and 2008 could be due to the fact that, during rainfall deficit periods, conservation tillage improves soil moisture and facilitates nutrients uptake.

Considering that Al concentration in maize tissues from the studied plots exceeded 6-8 times the threshold toxicity of 13 mg g^{-1} that led to reduced root length (Lidon and Barreiro, 1998), the low maize production we found (Gomez-Rey et al., 2013) was likely due to a problem of Al toxicity. Despite having Al levels similar or higher than those of maize, the good ryegrass yields suggested that this crop did not suffer Al toxicity under the studied conditions.

According with Benton-Jones (1991) criteria, most plant samples analysed (93 % and 83 % for ryegrass and maize, respectively) had a "sufficient" Fe concentration that was "high" for the rest of them. Consequently, the nutritive value of the fodder obtained was adequate for this element.

While Mn concentrations in all maize samples were well within the "sufficient" range reported by Benton-Jones (1991), they were "high" in all analysed ryegrass material, although rarely reached the threshold (140-300 mg kg^{-1}) that indicate growth depressions in cereals because of soil acidity (Zorn and Prausse, 1993). Therefore, the supply of Mn to crops can be considered satisfactory from the yield and nutritive point of views.

A "low" Cu concentration was a common fact for 94 % of ryegrass and 100 % of maize samples, with the remaining 6 % of ryegrass samples having a "sufficient" level of this micronutrient (see Benton-Jones, 1991). These results suggested that Cu deficiency could have a detrimental effect on ryegrass and maize production in the studied plots.

The different crop requirements led to contrasting patterns on Zn nutrition: 80 % of ryegrass samples had "high" Zn concentrations, that were "low" in 61 % of maize samples accordingly with the ranges indicated by Benton-Jones (1991), with 20 % (ryegrass) to 39 % (maize) of samples having a "sufficient" Zn concentration. Taking into account this difference between the crops of the studied rotation and knowing that Zn is one of main limiting factors of maize growth and yielding, a zinc foliar application to maize must be recommended (Potarzycki and Grzebisz, 2009). This recommendation is especially important because all maize samples had "low" Cu concentration and a synergism was observed between combined deficiency of Cu and Zn (Agarwala et al., 1995).

5. Conclusions

After 14 years under conservation and ploughing tillage, the tillage system explained only a limited fraction (3.5 to 11 %) of trace element availability. For Al, Fe, Mn and Cu no significant differences between tillage systems were found in the uppermost soil layer, while in the subsurface layers the highest values were always observed in the conventional tillage plots. Conversely, for Zn the highest value in the 0-5 cm soil layer was recorded under conservation tillage and there were no significant differences in the other soil layers. Results suggested that tillage effects on maize uptake of trace element are related with water availability during the growing season. From a trace elements point of view, no differences in crop nutritive value due to tillage system would be expected under the studied conditions of climate, soil, tillage and crop rotation. The low maize yields are likely due to the high soil Al availability (partially alleviated under conservation tillage), that led to plant concentrations up to 8 times the threshold toxicity, or to the low levels of available Zn and Cu. Despite the good production, results suggested that Cu deficiency could have a detrimental effect on ryegrass yields.

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